

Serial No. 10/706,296
Attorney Docket No. 632898-044
Amendment

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AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for purifying glycosides of genistin and daidzin from impurities present in a soy isoflavones concentrate, comprising the steps of:
 - (a) digesting a soy isoflavones concentrate with an acidic solution; and
 - (b) separating insoluble solids from the acidic solution, wherein said solids are ~~enriched in genistin and~~ comprise glycosides of genistin and daidzin and the ratio of genistin to daidzin is greater than about 3:1.
2. (Original) The method of claim 1 wherein the ratio of soy isoflavone concentrate to the acidic solution is between about 3:1 and 10:1 by weight.
3. (Original) The method of claim 1 wherein step (a) is conducted at a temperature of between about 10 and 100°C.
4. (Original) The method of claim 3 wherein step (a) is conducted at a temperature of between about 18 and 25°C.
5. (Original) The method of claim 1 wherein the acidic solution comprises glacial acetic acid or formic acid.
6. (Original) The method of claim 5 wherein the acidic solution comprises glacial acetic acid and an organic co-solvent selected from the group consisting of alcohols containing 1 to 12 carbons, aliphatic hydrocarbons containing 5 to 20 carbons, aromatic

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hydrocarbons containing 6 to 30 carbons, ketones containing 2 to 12 carbons, esters containing 3 to 30 carbons and mixtures thereof.

7. (Original) The method of claim 1 wherein the acidic solution comprises a mineral acid and an alcoholic co-solvent.
8. (Original) The method of claim 7 wherein said mineral acid is selected from the group consisting of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid and mixtures thereof and said alcoholic co-solvent is selected from the group consisting of alcohols containing 1 to 8 carbons and mixtures thereof.
9. (Original) The method of claim 8 wherein said acidic solution comprises hydrochloric acid and methanol.
10. (Original) The method of claim 9 wherein said acidic solution comprises hydrochloric acid and methanol in a volume ratio of about 1:5.
11. (Currently Amended) A method of preparing aglycons of genistin and daidzin comprising the steps of:
 - (a) digesting a soy isoflavone concentrate with a first acidic solution;
 - (b) separating insoluble solids wherein said solids comprise glycosides of genistin and daidzin and the ratio of genistin to daidzin is greater than about 3:1; and
 - (c) converting said glycosides to aglycons.
12. (Original) The method of claim 11 wherein step (c) comprises acidic or enzymatic hydrolysis.
13. (Original) The method of claim 12 wherein said acidic hydrolysis comprises refluxing said solids in a second acidic solution.
14. (Original) The method of claim 13 further comprising:

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(d) separating insoluble solids from the second acidic solution, wherein the insoluble solids separated from the second acidic solution are enriched in genistin and comprise aglycons of genistin and daidzin.

15. (Original) The method of claim 11 further comprising the following steps after step (c):

(i) dissolving said aglycons in an alkaline aqueous solution to obtain an alkaline aqueous solution containing dissolved aglycons;

(ii) separating insoluble impurities from said alkaline aqueous solution;

(iii) acidifying said alkaline aqueous solution to precipitate the aglycons as insoluble solids; and

(iv) separating said insoluble solids from said acidified solution, wherein the insoluble solids removed from said acidified solution are enriched in genistin and comprise aglycons of genistin and daidzin.

16. (Original) The method of claim 15 wherein said insoluble solids consist essentially of aglycons of genistin and daidzin.

17. (Original) The method of claim 15 wherein the pH of the alkaline aqueous solution is between about 10 and 14 and wherein the alkaline aqueous solution includes at least one primary cation selected from the group consisting of sodium, potassium, calcium, and ammonium.

18. (Original) The method of claim 15 wherein said acidifying step comprises adjusting the pH of the solution to a pH value of between about 1 and about 7 with a mineral acid selected from the group consisting of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, and mixtures thereof.

19. (Original) The method of claim 15 wherein the alkaline aqueous solution in step (i) has a pH of from about 10.5 to about 11.5, the acidified solution of step (iii) has a pH

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of from about 1 to about 4, and where steps (i) through (iv) are performed at temperatures below about 35°C.

20. (Original) The method of claim 11 wherein the ratio of soy isoflavone concentrate to the first acidic solution is between about 3:1 and 10:1 by weight.
21. (Original) The method of claim 11 wherein step (a) is conducted at a temperature of between about 10 and 100°C.
22. (Original) The method of claim 11 wherein the first acidic solution comprises glacial acetic acid or formic acid.
23. (Original) The method of claim 15 wherein at least one of the first and second acidic solutions comprises a mineral acid and an alcoholic co-solvent.
24. (Original) The method of claim 23 wherein said mineral acid is selected from the group consisting of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid and mixtures thereof and said alcoholic co-solvent is selected from the group consisting of alcohols containing 1 to 8 carbons and mixtures thereof.
25. (Original) The method of claim 24 wherein said mineral acid comprises hydrochloric acid and said co-solvent comprises methanol.
26. (Original) The method of claim 25 wherein said hydrochloric acid and methanol are present in a volume ratio of about 1:5.
27. (Original) A method of purifying glycosides of genistin, daidzin, and glycerin from impurities present in a soy isoflavone concentrate comprising the steps of:
(a) digesting a soy isoflavone concentrate with an acidic solution comprising glacial acetic acid in conjunction with an organic solvent that reduces the polarity of glacial acetic acid, thereby increasing the solubilities of daidzin and glycerin; and

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(b) separating the insoluble solids from said acidic solution, wherein said solids comprise glycosides of genistin, daidzin, and glycerin.

28. (Original) The method of claim 27 wherein said solids consist essentially of genistin, daidzin, and glycerin.

29. (Original) The method of claim 27 wherein said organic solvent is selected from the group consisting of alcohols containing 1 to 12 carbons, aliphatic hydrocarbons containing 1 to 20 carbons, aromatic hydrocarbons containing 6 to 30 carbons, ketones containing 2 to 12 carbons, esters containing three to 30 carbons, and mixtures thereof.